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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A23G 9/00, 9/02, A23L 3/37

A1

(11) International Publication Number: **WO 98/41107**

(43) International Publication Date: 24 September 1998 (24.09.98)

(21) International Application Number: PCT/EP98/01576

(22) International Filing Date: 12 March 1998 (12.03.98)

(30) Priority Data:
97301719.7 14 March 1997 (14.03.97) EP
(34) Countries for which the regional or international application was filed: GB et al.

(71) Applicant *(for all designated States except AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG)*: UNILEVER N.V. [NL/NL]; Weena 455, NL-3013 AL Rotterdam (NL).

(71) Applicant *(for AU BB CA GB IE KE LK LS MN MW NZ SD SG SZ TT UG only)*: UNILEVER PLC [GB/GB]; Unilever House, Blackfriars, London EC4P 4BQ (GB).

(72) Inventor: SMALLWOOD, Keith; Unilever Research Colworth, Colworth House, Sharnbrook MK44 1LQ (GB).

(74) Agent: UNILEVER N.V.; Patent Division, P.O. Box 137, NL-3130 AC Vlaardingen (NL).

(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: FROZEN FOOD PRODUCT

(57) Abstract

Use of storage temperatures of from –2 °C to –12 °C for frozen food products containing anti-freeze proteins.

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Frozen Food product

Technical Field of the Invention

The invention relates to a method of storing food product containing AFPs and to food products containing AFPs.

Background to the Invention

Anti-freeze peptides (AFPs) have been suggested for improving the freezing tolerance of foodstuffs.

For the purpose of this invention the term AFP has the meaning such as well-known in the art, see for example "Antifreeze proteins and their potential use in frozen food products", Marilyn Griffiths et al. Biotechnology Advances, Vol.13, pp.375-402, 1995.

WO 90/13571 discloses antifreeze peptides produced chemically or by recombinant DNA techniques from plants. The AFPs can suitably be used in food-products such as ice-cream.

WO 92/22581 discloses AFPs from plants which can be used for controlling ice crystal growth in ice-cream. This document also describes a process for extracting a polypeptide composition from intercellular spaces of plants by infiltrating leaves with an extraction medium without rupturing the plant cells.

WO 94/03617 discloses the production of AFPs from yeast and their possible use in ice-cream. WO 96/11586 describes fish AFPs produced by microbes.

WO 96/39878 suggests the production of AFP containing frozen compositions by leaving out the hardening step during the freezing process. Suitable storage temperatures are from -10 to -20°F (-23.3 - -28.9°C) and may even be as high as 10°F (-12.2°C).

Up till now, however the use of AFPs has not been applied to commercially available food products. One reason for this is that up till now it has proved difficult to reproducibly produce a frozen food product having the desired texture and eating characteristics.

The present invention aims at providing solutions to these problems. In particular the invention aims at providing frozen food products with improved mouthfeel and/or taste. Furthermore the invention provides a convenient and cheap process for storing frozen food products containing AFPs.

Surprisingly it has been found that AFPs can conveniently be incorporated in frozen food products to result in the desired product and process properties if the frozen food product is after preparation stored at a moderate freezing temperature.

Accordingly in a first aspect the invention relates to a method of storing a frozen food product containing AFPs after preparation, whereby the storage temperature is maintained at from -2.0°C to -12.0°C.

Although applicants do by no means wish to be bound by any theory it is believed that the explanation of the invention is as follows: If food products without AFPs are stored for prolonged periods at relatively high temperatures, normally relatively large ice-crystals are formed throughout the product leading to unacceptable eating characteristics. To avoid this, frozen food products are usually stored at relatively low temperatures say -18°C or lower. A disadvantage of these storage temperatures is however that the frozen products are relatively hard and have a less than optimal flavour release in the mouth if they are eaten at the storage temperature. Surprisingly it has been found that if AFPs are included in food products to be frozen this generally leads to a favourable change in ice-recrystallisation properties allowing the storage of the frozen product at relatively high temperatures and thereby improving the texture and taste if they are eaten at storage temperatures.

Frozen products according to the invention have and improved texture and flavour e.g. better creaminess, smoothness, warmth of eating, improved aroma, flavour release etc.

Another advantage of relatively high storage temperatures is that freezers can be used with a lower energy consumption and with a reduced tendency of ice-formation in the freezer.

Frozen food products of the invention may be any food product which can be stored and/or eaten in the frozen

state. Examples of frozen food products which may contain AFP are processed food products such as for example frozen bakery products e.g. doughs, batters, cakes etc., frozen culinary products for example soups, sauces, pizzas, frozen vegetable products such a compote, mashed potato, tomato paste etc. A very preferred food product according to the invention is a frozen confectionery product.

For the purpose of the invention the term frozen confectionery product includes milk containing frozen confections such as ice-cream, frozen yoghurt, sherbet, sorbet, ice milk and frozen custard, water-ices, granitas and frozen fruit purees. Especially preferred products of the invention are ice-cream and water-ice.

Preferred storage temperatures of the frozen food are from -6.0°C to -12.0°C., most preferred from -10.0°C to -12.0°C.

The storage temperature of the frozen food refers to the temperature at which the product is maintained after final preparation. A convenient process for the preparation of a frozen product of the invention, especially a frozen confectionery product involves the mixing of the ingredients at a relatively high temperature (e.g. above the freezing point of water, for example at ambient temperature) followed by cooling and freezing. During freezing (optional) aeration may take place e.g. to an overrun of 50 to 250%. Generally the freezing involves a prefreezing step, e.g. in an scraped surface heat exchanger e.g. to a temperature of from -2 to -10°C, followed by filling the prefrozen product into containers followed by

optional hard-freezing e.g. to the storage temperature of the invention.

Frozen products of the invention can be stored at the temperature of the invention for prolonged periods e.g. from 1 day to 5 years, mostly somewhere between 1 week and 6 months, mostly 2-10 weeks. Suitably frozen products are stored in a freezer cabinet which is maintained at the temperature of the invention. These freezer cabinets can be any freezing cabinet which is used for the storage of frozen food products e.g. in shops or in mobile freezing cabinets. In shops generally the freezer cabinets are display cabinets, whereby either part of the cabinet is transparent or part of the cabinet is open to allow a view of the frozen products.

Applicants have found that the AFPs for use in the process of the invention can come from a variety of sources. One possible source of AFP materials is fish. Examples of fish AFP materials are AFGP (for example obtainable from Atlantic cod, Greenland cod and Tomcod), Type I AFP (for example obtainable from Winter flounder, Yellowtail flounder, Shorthorn sculpin and Grubby sculpin), Type II AFP (for example obtainable from Sea raven, Smelt and Atlantic herring) and Type III AFP (for example obtainable from Ocean out, Atlantic wolffish, Radiated shanny, Rock gunnel and Laval's eelpout). A preferred example of the latter type is described in WP 97/02343.

Another possible source of AFP material are invertebrates. Also AFPs may be obtained from Bacteria.

A third possible source of AFP material are plants. Examples of plants containing AFPs are garlic-mustard, blue wood aster, spring oat, winter cress, winter canola, Brussels sprout, carrot, Dutchman's breeches, spurge, daylily, winter barley, Virginia waterleaf, narrow-leaved plantain, plantain, speargrass, Kentucky bluegrass, Eastern cottonwood, white oak, winter rye, bittersweet nightshade, potato, chickweed, dandelion, spring and winter wheat, triticale, periwinkle, violet and grass.

Both natural occurring species may be used or species which have been obtained through genetic modification. For example micro-organisms or plants may be genetically modified to express AFPs and the AFPs may then be used in accordance to the present invention.

Genetic manipulation techniques may be used to produce AFPs as follows: An appropriate host cell or organism would be transformed by a gene construct that contains the desired polypeptide. The nucleotide sequence coding for the polypeptide can be inserted into a suitable expression vector encoding the necessary elements for transcription and translation and in such a manner that they will be expressed under appropriate conditions (e.g. in proper orientation and correct reading frame and with appropriate targeting and expression sequences). The methods required to construct these expression vectors are well known to those skilled in the art.

A number of expression systems may be utilised to express the polypeptide coding sequence. These include, but are not limited to, bacteria, yeast insect cell systems, plant cell

culture systems and plants all transformed with the appropriate expression vectors.

A wide variety of plants and plant cell systems can be transformed with the nucleic acid constructs of the desired polypeptides. Preferred embodiments would include, but are not limited to, maize, tomato, tobacco, carrots, strawberries, rape seed and sugar beet.

For the purpose of the invention preferred AFPs are derived from fish or plants. Especially preferred is the use of fish proteins of the type III, most preferred HPLC 12 as described in our WO 97/02343. Another preferred AFP is derived from carrots such as described in our non-prepublished case PCT/EP97/06181 or from grass as disclosed in our non-prepublished PCT/EP97/03634.

For some natural sources the AFPs may consist of a mixture of two or more different AFPs.

Preferably those AFPs are chosen which have significant ice-recrystallisation inhibition properties. This can be measured in accordance to the method of Example III.

Preferably AFPs in accordance to the invention provide an ice particle size upon recrystallisation -as measured in accordance to the examples- of less than 20 µm, more preferred from 5 to 15 µm.

Preferably the level of solids in the frozen food product (e.g. sugar, fat, flavouring etc.) is more than 2 to 3 wt%, more preferred from 4 to 70 wt%.

Very conveniently the products of the invention are packed in closed containers (e.g. cartons, bags, wrappers, boxes, plastic containers etc.). For single portions the pack size will generally be from 10 to 1000 g. For multiple portions pack sizes of up to 500 kg may be suitable. Generally the pack size will be from 10 g to 5000 g.

As indicated above the preferred products wherein the AFPs are used are frozen confectionery product such as ice-cream or water-ice. Preferably the level of AFPs is from 0.0001 to 0.5 wt% based on the final product, more preferred 0.01 to 0.4 wt%, most preferred 0.05 to 0.3 wt%.

The aspect ratio of ice-crystals in compositions which are stored according to the invention is preferably maintained at a level of less than 2.0, e.g. from 1.0 to 1.9. The aspect ratio of ice-crystals is defined as the average ratio of the length and the breadth of the ice-crystals. An aspect ratio of less than 2.0 corresponds to roundish ice-crystals, which are not elongated in shape. The aspect ratio of crystals can be determined by any suitable method.

Example I

A pre-mix for preparing ice-cream was made by mixing:

Ingredient	% by weight		
	A	B	C
Skimmed milk powder		10.00	
sucrose		13.00	
maltodextrine (MD40)		4.00	
Locust bean gum		0.14	
butter oil		8.00	
monoglyceride (palmitate)		0.30	
vanillin		0.01	
AFP (Type III HPLC-12)	0.01	0.01	none
water		balance	

The mixes can be used in the preparation of a ice-cream by homogenisation at 2000 psi and 65°C followed by ageing over night at 5 C. The mix is frozen using a freezer (MF50 SSHE Technohoy fitted with a solid dasher rotating 240 rpm) The extrusion temperature is -4.5°C, the overrun is 110%. Products A and C are then frozen at -12.0°C and stored for 3 weeks. Product B is frozen at -18°C for 3 weeks. The products are tasted directly after removing from the freezer.

After 3 weeks storage composition A according to the invention had a markedly better taste and creaminess than the control sample B. Also composition A had a markedly better texture than composition C.

Example II

An ice-cream was prepared of the following formulation:

Ingredient	% by weight
Skimmed milk powder	10.00
sucrose	13.00
maltodextrine (MD40)	4.00
Locust bean gum	0.14
butter oil	8.00
monoglyceride (palmitate)	0.30
vanillin	0.01
AFP (Carrot AFP)	0.01 or none(control)
water	balance

The method of preparation was as in example I.

Carrot AFP was added in the form of an amount of carrot juice obtained from cold acclimatised winter carrots as follows: Root extract from cold acclimatised carrot roots was prepared by scrubbing freshly pulled cold acclimatised carrots in cold water. The tops are removed and the juice extracted employing a domestic juice extractor (Russell Hobbs, model no 9915). The juice was frozen in 1 litre blocks and stored a -20°C prior to collection for use in ice cream trials.

The amount of carrot juice was chosen such that the amount of carrot AFP (the protein having an apparent molecular weight on SDS-PAGE of 38 kDa showing ice-recrystallisation properties) corresponds to 0.01 wt%.

After two months storage at -10.0°C the composition according to the invention had a markedly better texture and flavour than the control sample. Similarly the composition stored at -10.0°C had a better flavour than the same composition which was stored and tasted at -18.8°C.

Example III

Method of determining whether an AFP possesses ice recrystallisation inhibition properties.

Recrystallisation inhibition properties can measured using a modified "splat assay" (Knight et al, 1988). 2.5 µl of the solution under investigation in 30% (w/w) sucrose is transferred onto a clean, appropriately labelled, 16 mm circular coverslip. A second coverslip is placed on top of the drop of solution and the sandwich pressed together between finger and thumb. The sandwich is dropped into a bath of hexane held at -80°C in a box of dry ice. When all sandwiches have been prepared, sandwiches are transferred from the -80°C hexane bath to the viewing chamber containing hexane held at -6°C using forceps pre-cooled in the dry ice. Upon transfer to -6°C, sandwiches can be seen to change from a transparent to an opaque appearance. Images are recorded by video camera and grabbed into an image analysis system (LUCIA, Nikon) using a 20x objective. Images of each splat are recorded at time = 0 and again after 30-60 minutes. The size of the ice-crystals in both assays is compared. If the size at 30-60 minutes is similar only moderately (less than 10%) increased compared to the size at t=0, this is an indication of good ice-crystal recrystallisation properties.

Also if the ice-crystal size number (average length) is less than 20 µm, preferably from 5-15 µm this is a sign of good ice-crystal recrystallisation inhibition properties. The ice-crystal size can conveniently be determined by

highlighting the crystals manually and drawing around the perimeter. Images of the highlighted crystals can then be measured using image analysis software which counts the number of pixels to complete the longest straight line (length), shortest straight line (breadth) and the aspect ratio (length/breadth). The number average length is used as particle size.

Claims

1. A method of storing a frozen food product containing AFPs after preparation, whereby the storage temperature is maintained at from -2.0°C to -12.0°C.

2. A method according to claim 1, wherein the storage temperature is from -10.0°C to -12.0°C

3. A method according to claim 1, wherein the frozen food product is a frozen confectionery product comprising from 0.0001 to 0.5 wt% of AFPs.

4. A method according claim 1, wherein the product is stored at a temperature from -2.0°C to -12.0°C for a period of 1 day to 5 years.

5. A method according to claim 4, wherein the product is stored at a temperature from -2.0°C to -12.0°C for a period of 1 week to 6 months.

6. Frozen food product stored according to the method of claim 1.

7. Frozen food product according to claim 6 being a frozen confectionery product.

8. Frozen food product according to claim 6 wherein the AFP is derived from plants.

9. Frozen food product according to claim 6 packed in closed containers wherein the pack size is from 10 g to 500 kg.

INTERNATIONAL SEARCH REPORT

national Application No
PCT/EP 98/01576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23G9/00 A23G9/02 A23L3/37

According to International Patent Classification(IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23G A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 39878 A (THE PILLSBURY COMP.) 19 December 1996	1-7,9
Y	see page 6, line 7 - line 31; claims 1,2,6,8; examples 1,2	8
Y	WO 97 02343 A (UNILEVER) 23 January 1997 see the whole document	8
X	R. E. FEENEY ET AL.: "antifreeze proteins" FOOD TECHNOLOGY, vol. 47, no. 1, 1993, CHICAGO, pages 82-90, XP002040501	1,3,6,7
A	see page 87, column 1, paragraph 2 - column 2, paragraph 1	8

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[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex.

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Date of the actual completion of theinternational search	Date of mailing of the international search report
22 July 1998	29/07/1998

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International Application No
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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	WO 94 03617 A (UNILEVER) 17 February 1994 see abstract	1,8
X,P	GB 2 315 753 A (UNILEVER) 11 February 1998 see claims; examples	1-4,6-8

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INTERNATIONAL SEARCH REPORT

Information on patent family members

I National Application No
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